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Augments the Tumoricidal Activity of Antibodies or
Vaccines used for the Immunotherapy of Breast Cancer

PRINCIPAL INVESTIGATOR: Gordon D. Ross, Ph.D.

CONTRACTING ORGANIZATION: University of Louisville Research
Foundation, Inc.
Louisville, KY 40292-0001

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Annual Report for Award Number DAMD17-02-1-0445

INTRODUCTION

The objective is to investigate the use of orally administered barley β -glucan as an adjuvant for the immunotherapy of breast cancer to be used in combination with anti-tumor antibodies or vaccines. Previous research had shown that β -glucan isolated from barley or yeast bound to the leukocyte iC3b-receptor, CR3, generating a primed state that allowed the receptor to stimulate the killing of tumor cells coated with the CR3 ligand iC3b (1-3). Therapy of murine mammary tumors with intravenous yeast β -glucan had shown a requirement for anti-tumor antibody that activated complement and deposited iC3b on tumor cells (4). Therapy failures in mice deficient in either leukocyte CR3 or serum C3 highlighted the requirement for iC3b on tumors and CR3 on leukocytes. Because of the difficulty of obtaining quantities of soluble yeast β -glucan needed for human clinical trials, experiments were carried out with barley β -glucan that was available in quantity and appeared to have CR3-priming activity similar to yeast β -glucan. Initial research by the consultant to this project, Dr. Nai-Kong Cheung, demonstrated that orally administered barley β -glucan could be effective against human tumors implanted in nude or SCID mice, provided that anti-tumor antibodies were administered simultaneously by intravenous injection (5,6). The aims of this proposal were to determine the mechanism of action of orally administered barley β -glucan and whether it could be an effective adjuvant for antibody therapy in the same way as intravenously administered yeast β -glucan. It was hypothesized that the orally administered β -glucan crossed the gastrointestinal track and primed the CR3 of circulating leukocytes to recognize and kill iC3b-opsonized breast tumor cells.

BODY

At an early stage of the research, a small company was identified, Biopolymer Engineering, Inc., of Eagan, Minnesota, that could supply yeast β -glucan of high quality and in quantities that would be required for clinical trials. Because yeast β -glucan had been previously shown to have a higher affinity for CR3 than did barley β -glucan (1), it appeared possible that the yeast β -glucan might prove to be superior to the barley β -glucan as an adjuvant for tumor immunotherapy. However, yeast β -glucan had never been shown to function following oral administration in the same way as the barley β -glucan and therefore the barley β -glucan might prove to be a better therapeutic drug if it could be administered orally rather than intravenously. Accordingly, the decision was made to test the barley β -glucan and yeast β -glucan in parallel to determine which might be better as an adjuvant and also whether the yeast β -glucan might be able to function as an oral therapeutic in the same way as the barley β -glucan. Thus, the experiments with the yeast β -glucan were done in addition to, and not instead of the experiments with barley β -glucan that had been listed in the approved Statement of Work.

Two forms of yeast β -glucan were available from Biopolymer Engineering: 1) a soluble preparation for intravenous administration and 2) an insoluble particulate for possible oral administration. Both of these yeast β -glucan preparations, one given intravenously and the other given orally, were compared to the barley β -glucan administered orally.

Progress on the tasks listed in the Statement of Work:

Task 1. Determine if mouse blood and peritoneal neutrophil CR3 is primed for cytotoxicity following administration of barley β -glucan by intragastric injection - Years 1 and 2

a. Determine the optimal time required for priming: Months 1-6, year 1

Using β -glucans labeled with fluorescein, it was possible to follow the fate of ingested β -glucan-fluorescein by fluorescence microscopy and flow cytometry of isolated mouse tissues. With either barley or yeast β -glucan given by intragastric injection, 3 days were required before macrophages appeared in the

spleen that contained ingested fluorescein-labeled β -glucan. Macrophages are known to line the gastrointestinal track of the small intestine, and these were shown to be the cells responsible for the oral uptake of β -glucans. Three days were required for the β -glucan-containing gastrointestinal macrophages to migrate to the spleen and 4 days were required for the macrophages to appear in bone marrow removed from femurs. Following ingestion of the β -glucan, the macrophages broke down the large barley β -glucan into smaller β -glucan fragments that were shed from the macrophages and became bound to the membrane CR3 of both macrophages and the marginated stores of neutrophils contained in bone marrow. CR3 was not required for uptake of the β -glucan by intestinal macrophages, as fluorescently-labeled β -glucan was detected in macrophages from both wild-type and CR3-deficient mice in approximately equal proportions. However, the shed fluorescently-labeled β -glucan fragments did not bind to the membrane surface of CR3-deficient macrophages or neutrophils. Both the yeast and barley β -glucan were shown to prime the CR3 of splenic macrophages and peritoneal neutrophils elicited with thioglycolate. However, neutrophils with primed CR3 were not detected in peripheral blood unless mice were treated with intravenous endotoxin (LPS) that triggered the release of the marginated pool of bone marrow neutrophils into the blood. Alternatively, the primed marginated bone marrow neutrophils could be recruited to the peritoneal cavity by an overnight treatment of mice with intraperitoneal thioglycolate. Thus, neutrophils with primed CR3 are not normally found in any measurable quantity in the blood, and are recruited directly from the bone marrow into tumors with minimal time in transit within the blood. Tumor recruitment of neutrophils was shown to require complement activation within the tumor that released the chemotactic factor C5a. The therapeutic tumoricidal response of either barley or yeast β -glucan was completely inhibited in mice treated with a cyclic peptide antagonist of the C5a-receptor.

The optimal time required to detect primed neutrophils in the peritoneal cavity or blood was 6-7 days, at which time the majority of macrophages in the marrow were releasing soluble β -glucan that bound to the membrane CR3 of marrow neutrophils. However, primed neutrophils were only readily detectable in the blood if mice were treated with LPS that triggered the release the marginated pool of marrow neutrophils into the blood. Thus, it may not be possible to detect neutrophils with primed CR3 in the blood of human volunteers who ingest the barley β -glucan, since the approved human studies protocol does not include treatment with an agent that would release the marginated pool of neutrophils from the bone marrow. Although administration of LPS would not be safe in human volunteers, it would be possible to administer a low dose of the cytokine G-CSF to volunteers in the same way as is done to mobilize stem cells and marginated neutrophils into the blood for bone marrow transplantation. Such a change in the human studies protocol is being considered but has not yet been proposed.

b. Determine optimal dose of barley β -glucan for priming: Months 7-12, year 1

The optimal daily dose of barley or yeast β -glucan was determined by intragastric injection of tumor-bearing mice that were being simultaneously treated with intravenous infusions of anti-tumor monoclonal antibody. With either source of β -glucan, the optimal dose that produced the most tumor regression was 200-400 μ g per day. Treatment of mice with this range of β -glucan doses produced the maximum tumor regression activity as compared to mice that received doses of 50, 100, or 800 μ g per day. Based on a mouse body weight of 25 grams, the calculated optimal dose for a 75,000 gram (165 pound) human patient would be 600 to 1200 mg per day. Phase I trials carried out by Biopolymer Engineering have indicated no side effects with doses of oral yeast β -glucan of as high as 15 grams per day for 6 weeks.

c. Determine the correlation between in vitro and in vivo priming activity: Months 7-12, year 1

In vitro priming with soluble barley or yeast β -glucan was found to be nearly instantaneous with human or mouse neutrophils. However, there was a 3 day delay for in vivo priming of neutrophils following oral administration of particulate yeast β -glucan, and a 2-3 day delay in priming for oral barley β -glucan.

The delay with the oral β -glucans appeared to be the time required for gastrointestinal macrophages to transport the β -glucan to the margined pool of neutrophils in the bone marrow and then break down the large β -glucans into small β -glucan fragments that were capable of priming neutrophils in the bone marrow. As expected, the time required for in vivo priming of blood neutrophils by soluble yeast β -glucan administered intravenously was also nearly instantaneous, since the β -glucan was presented to the blood neutrophils directly in a usable form and did not require transport or processing by macrophages. Based on these results, tumor immunotherapy protocols were revised so that the oral yeast or barley β -glucan was administered three days before administering the intravenous anti-tumor mAb. When this was done, the kinetics of in vivo tumor regression (tumor diameter versus time) obtained with the oral β -glucans was the same as that obtained when soluble intravenous yeast β -glucans were administered at the same time as the anti-tumor mAb. An important finding from these in vivo experiments was that the oral yeast β -glucan was significantly better in producing tumor regression and long-term tumor-free survival than was the oral barley β -glucan (Fig. 1).

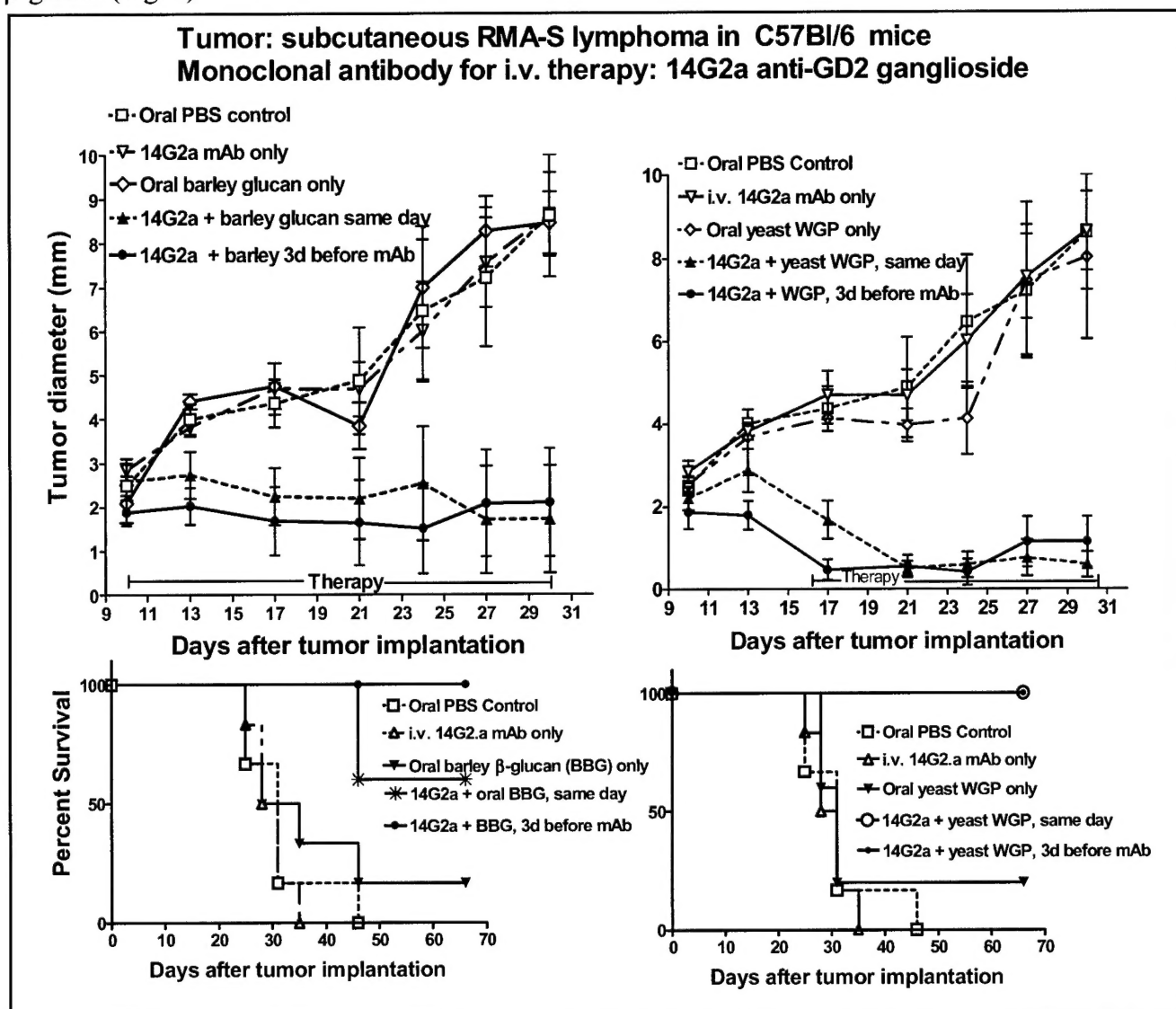


Figure 1. Both barley β -glucan and yeast β -glucan (whole glucan particles, WGP) produced tumor regression when given orally in combination with intravenous anti-tumor monoclonal antibody (mAb). Tumor regression and long-term tumor-free survival were better in the groups of mice treated with mAb plus oral yeast rather than barley β -glucan. Both β -glucans gave better regression when started 3-days before the mAb therapy. All mice treated with mAb plus oral yeast WGP β -glucan were long-term tumor-free survivors.

d. Determine correlation between priming activity and tumor regression: Months 7-12, year 1.

The optimal dose of both barley β -glucan and yeast β -glucan for in vivo priming was found to be 400 μ g per day, with maximal priming detected after 7 days. The 7-day time period appeared to correspond to the time required for the majority of bone marrow macrophages to become loaded with ingested β -glucan. Subsequently elicited peritoneal macrophages exhibited maximum cytotoxic activity against iC3b-opsonized tumor cells on day 7 after beginning daily intragastric injections of β -glucan. Tumor regression activity also appeared to be maximal on day 7 after starting intragastric β -glucan therapy (Fig. 1).

Task 3: Oral barley β -glucan therapy of mice with syngeneic mammary carcinoma - Years 1 and 2

a. Therapy of Ptas64 tumors in normal and CR3-KO BALB/c mice: Months 6-12, year 1; and months 1-6, year 2

These studies have been completed earlier than expected. The CR3-KO mice obtained from our collaborator, Dr. Tanya Mayadas-Norton of Harvard University, were provided on a C57Bl/6 background rather than on a BABL/c background. This required use of a C57Bl/6 syngeneic tumor rather than Ptas64 that will only form tumors in BALB/c mice. The RMA-S tumor was selected because of the availability of a monoclonal antibody that recognized a highly expressed tumor antigen expressed by RMA-S cells, GD2 ganglioside. Dr. Ralph Reisfeld, Research Institute of Scripps Clinic, La Jolla, CA, generously provided us with his 14.G2a hybridoma that secretes mouse IgG2a anti-GD2 ganglioside mAb, and pilot studies demonstrated that this mAb was very effective in opsonizing the RMA-S tumor cells with iC3b in vivo after implantation of the tumor cells in a mammary fat pad of C57Bl/6 mice. As had been shown earlier with intravenous yeast β -glucan therapy (4), the tumor regression activity of orally administered barley or yeast β -glucans were nearly absent in CR3-KO C57Bl/6 mice as compared to wild type mice. The ability of the oral β -glucans to elicit long-term tumor-free survival was also severely compromised in the CR3-KO mice as compared to wild type mice (Fig. 2).

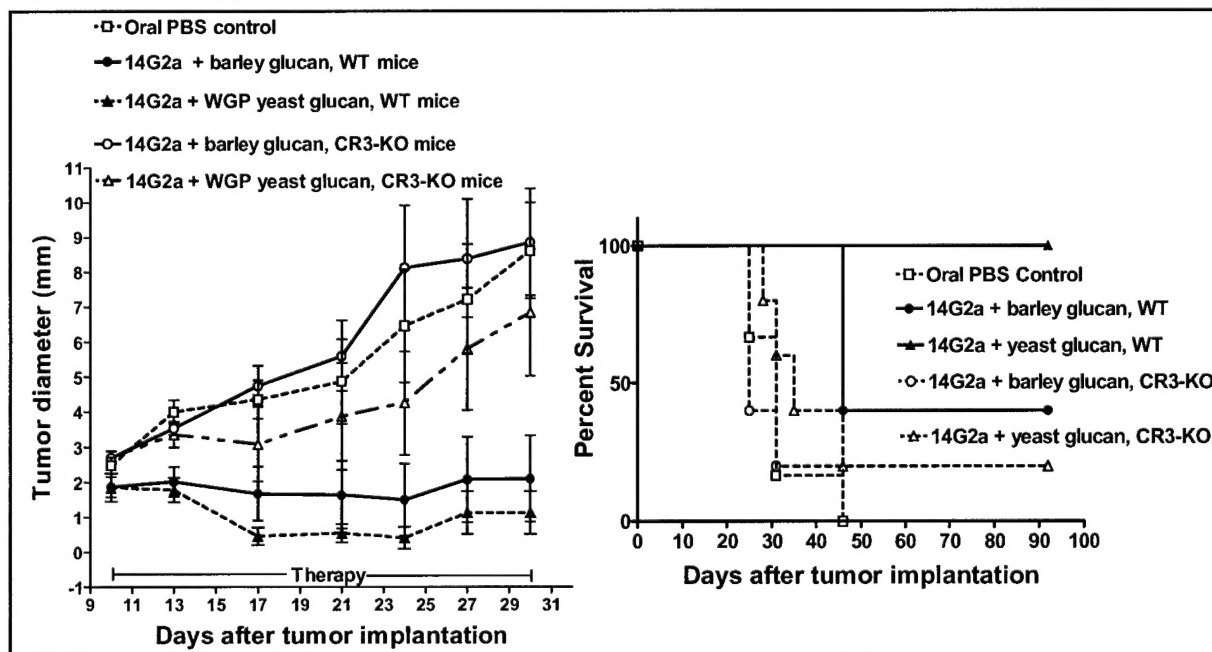


Figure 2. Oral β -glucan adjuvant activity with either barley or yeast β -glucan requires leukocyte CR3. Tumor regression activity (left graph) is significantly greater with yeast β -glucan as compared to barley β -glucan in wild-type (WT) mice ($p < 0.05$), and there is little tumor regression with either glucan in CR3-deficient (CR3-KO) mice. All WT mice treated with oral yeast β -glucan + 14G2a mAb were long-term tumor-free survivors versus only 40% of mice treated with 14G2a mAb + oral barley β -glucan (right side graph). Survival was also greatly reduced in CR3-KO mice, with no long-term survival in the mice receiving oral barley β -glucan + mAb and only 20% survival in the CR3-KO mice receiving mAb + oral yeast β -glucan.

b. Therapy of RMA-MUC tumors in normal and C3-KO C57Bl/6 mice: Months 4-12, year 2

Although these studies were scheduled for year 2 in the Statement of Work, we have begun these mouse tumor protocols and the experiments are nearly completed except for the analysis of tumor-free, long-term survival. The results indicated that the serum protein C3 is also required for the tumor regression response function of orally administered barley or yeast β -glucan. There was virtually no tumor regression response in C3-deficient (C3-KO) mice as compared to their wild-type littermates. This finding supports the central hypothesis of the grant proposal that the adjuvant effect of oral β -glucans requires that tumor cells be targeted with iC3b that is deposited onto tumors through the action of anti-tumor mAbs and the serum complement system.

Task 4: Monitor the uptake, pharmacokinetics, and biodistribution of oral ^{125}I -barley β -glucan - Years 2-3

a. Label various lots of purified barley β -glucan with tyramine and ^{125}I : Months 2-9, year 2

Research on the best method to label β -glucans for in vivo studies of uptake and biodistribution have indicated that a fluorescein label is superior to the previously proposed radioactive iodine label.

b. Validate the specific binding of ^{125}I -barley β -glucan by leukocyte CR3: Months 5-10, year 2

Research with barley β -glucan labeled with fluorescein have indicated that macrophage ingestion is not strictly dependent upon CR3, as it was observed with macrophages from CR3-deficient mice. However, much more barley β -glucan was bound to the outer membranes of wild-type as compared to CR3-deficient macrophages and neutrophils.

c. Monitor the uptake of oral ^{125}I - β -glucan into the blood and major organs: Months 9-12, year 2, and months 1-4, year 3

Studies have been completed with particulate yeast β -glucan labeled with fluorescein and studies are ongoing with fluorescein-labeled barley β -glucan. As mentioned above, these studies showed that the orally administered yeast whole β -glucan particles (WGP-F) were taken by gastrointestinal macrophages that transported the WGP-F to lymphoid tissues in the spleen, lymph nodes, and bone marrow. Within these lymphoid tissues, the macrophage used a mechanism of oxidative metabolism to break down the β -glucan particles into soluble β -glucan fragments that retained the fluorescence label. The soluble β -glucan-F was taken up by macrophages and neutrophils via their membrane CR3, and no membrane uptake was detectable with neutrophils from CR3-deficient mice (Fig. 3). The neutrophils and macrophages with surface-bound,

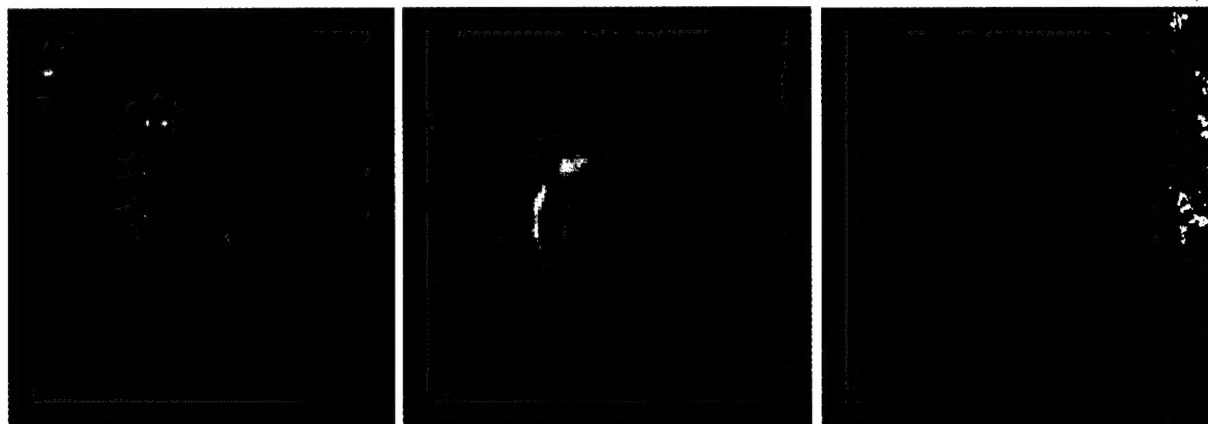


Figure 3. Bone marrow cells from mice that had been fed fluorescein-labeled yeast whole β -glucan particles (WGP-F) daily for 7 days. At first, intact WGP-F is detected only within macrophages identified by staining with F4/80-cychrome (red membrane stain; left panel). The WGP-F is digested over several days so that smaller β -glucan particles are later seen within macrophages (middle panel). Still later, neutrophils (that lack red membrane staining for macrophages) are observed bearing surface membrane bound soluble β -glucan (right panel).

fluorescein-labeled β -glucan remained in the bone marrow and did not circulate readily in the blood. Neutrophils containing surface-bound β -glucan were detected in the blood of mice that had been administered endotoxin (LPS) to release the marginated pool of neutrophils from the bone marrow into the blood. Other studies showed that neutrophils with surface-bound β -glucan were recruited from the bone marrow into tumors through activation of complement at the tumor site with release of the chemotactic factor C5a.

Task 5: Determine the approximate size of ^{125}I -labeled barley β -glucan in the blood and the specific organs found to accumulate ^{125}I following oral administration of ^{125}I -labeled barley β -glucan - Year 3

a. Analyze the size of ^{125}I - β -glucan in serum from mice after oral administration: Months 3-6, year 3

Research has now indicated that orally administered β -glucan is broken down by macrophages into smaller soluble fragments of approximately 20-80 kD that remain bound to blood neutrophils via CR3 and that there is no soluble β -glucan present in serum from mice after oral administration. The neutrophils carry the β -glucan on their membrane CR3 into tumors where the β -glucan functions to prime the CR3 to trigger the killing of tumors opsonized with the CR3 target ligand iC3b. At this point, these studies have been done only with yeast β -glucan, and experiments are ongoing to track orally administered barley β -glucan in vivo.

b. Analyze the size of ^{125}I - β -glucan in cell-free homogenates of organs after oral administration: Months 7-12, year 3

These studies are ongoing with fluorescein-labeled yeast β -glucan and will be started later with labeled barley β -glucan. The primary target organs for ingested β -glucan are bone marrow and spleen, and so these are the organs from which homogenates will be prepared for analysis of β -glucan size.

KEY RESEARCH ACCOMPLISHMENTS

Conclusions drawn from the research during the first year of study:

- The tumoricidal response to orally administered barley or yeast β -glucan requires the presence of iC3b on tumor cells and CR3 on leukocytes.
- Orally administered β -glucans are taken up by gastrointestinal macrophages in a CR3-independent manner and the macrophages shuttle the β -glucan to lymphoid tissues including spleen, lymph nodes and bone marrow.
- In the bone marrow, macrophages partially digest the β -glucan and secrete soluble fragments of β -glucan of approximately 20-80 kD in size.
- The soluble β -glucan released by macrophages in the bone marrow is taken up onto the membrane CR3 of macrophages and neutrophils. There is never any measurable soluble β -glucan in serum.
- Neutrophils and macrophages bearing β -glucan-primed membrane CR3 remain in the bone marrow until recruited to tumors by complement activation and the release of C5a at the tumor site.

REPORTABLE OUTCOMES

Two manuscripts are currently in preparation and data for a third manuscript should be completed by August. The manuscripts could not be submitted earlier because of the time required to show that β -glucan therapy of mice produced long-term, tumor-free survival (90 days per protocol). Presentations about the data were given at the Annual Meeting of the American Association of Immunologists held in Denver during May, 2003. The abstract for an additional presentation has been accepted for oral presentation at the 9th European Meeting on Complement in Human Disease to be held in Trieste, Italy from September 6-9, 2003.

Published abstracts of presentations:

1. Allendorf, D. J., J. T. Baran, R. D. Hansen, K. Subbarao, D. Walsh, F. Hong, J. Marroquin, J. Yan, J. D. Lambris, B. Haribabu, and G. D. Ross. 2003. Orally administered β -glucan functions via anti-tumor mAbs and the complement system to recruit CR3⁺ neutrophils and macrophages that produce tumor regression and tumor-free survival. *Mol. Immunol.*, in press (Abstract)
2. Allendorf, D. J., J. T. Baran, R. D. Hansen, F. Hong, J. Marroquin, J. Yan, D. Walsh, and G. D. Ross. 2003. Macrophages shuttle orally administered β -glucan to potentiate the CR3-dependent tumoricidal effects of monoclonal antibodies in mouse tumor models. *FASEB J.* 17:C128 (Abstract)
3. Ross, G. D., R. D. Hansen, D. J. Allendorf, J. T. Baran, J. Yan, K. Subbarao, B. Haribabu, and J. D. Lambris. 2003. Tumor regression mediated by β -glucans against iC3b-opsonized tumors requires CR3⁺ neutrophils recruited via C5a and leukotriene B₄ (LTB₄). *FASEB J.* 17:C128 (Abstract)

CONCLUSIONS

The work completed during the first year of this award, in addition to previous studies by us and others, provides strong evidence for the potential utility of an oral β -glucan adjuvant for use in combination with therapeutic anti-tumor monoclonal antibodies such as Herceptin, Rituxan, and Erbitux. The tumor regression activity of all three of these humanized monoclonal antibodies has been shown to be significantly increased by the simultaneous administration of oral β -glucan when tested in SCID mice bearing human tumor implants expressing the relevant tumor antigens. Vaccines that generate similar antibodies in patients could also benefit from simultaneous administration of oral β -glucan. The current data showing a requirement that the therapeutic antibodies activate complement and deposit iC3b on tumor cells is important because it means that therapeutic antibodies that have been engineered so that they do not activate complement would not benefit from co-administration of oral β -glucan. Through use of mice deficient in C3 or CR3, this research demonstrated that oral β -glucan therapy required that tumors be targeted with iC3b and for recognition by leukocytes bearing β -glucan-primed CR3. Neutrophils used their membrane CR3 for uptake of soluble β -glucan fragments released from gastrointestinal macrophages that had initially taken up either particulate yeast β -glucan or large soluble molecules of barley β -glucan. Oral yeast β -glucan was shown to elicit significantly better tumor regression and long-term tumor-free survival than did oral barley β -glucan. Since soluble β -glucan does not appear in the blood but remains bound to tissue macrophages or bone marrow neutrophils, the previously planned protocol to measure blood neutrophil priming in normal human subjects who have ingested barley β -glucan may not be successful. However, tests in mice may indicate that a revised human subject protocol should be considered, and this would necessitate another review both by the Army Human Studies Research Review Board and the University of Louisville IRB.

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3. Xia, Y., V. Vetvicka, J. Yan, M. Hanikyrova, T. N. Mayadas, and G. D. Ross. 1999. The β -glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.* 162:2281-2290.
4. Yan, J., V. Vetvicka, Y. Xia, A. Coxon, M. C. Carroll, T. N. Mayadas, and G. D. Ross. 1999. β -Glucan, a "specific" biologic response modifier that uses antibodies to target tumors for recognition by complement receptor type 3 (CD11b/CD18). *J. Immunol.* 163:3045-3052.

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APPENDIX

Photocopy of page from *FASEB Journal* containing the published abstracts for two presentations at the Annual Meeting of the American Association of Immunologists, Denver, CO, May, 2003:

1. Allendorf, D. J., J. T. Baran, R. D. Hansen, F. Hong, J. Marroquin, J. Yan, D. Walsh, and G. D. Ross. 2003. Macrophages shuttle orally administered β -glucan to potentiate the CR3-dependent tumoricidal effects of monoclonal antibodies in mouse tumor models. *FASEB J.* 17:C128 (Abstract)
2. Ross, G. D., R. D. Hansen, D. J. Allendorf, J. T. Baran, J. Yan, K. Subbarao, B. Haribabu, and J. D. Lambris. 2003. Tumor regression mediated by β -glucans against iC3b-opsonized tumors requires CR3⁺ neutrophils recruited via C5a and leukotriene B₄ (LTB₄). *FASEB J.* 17:C128 (Abstract)

Macrophages shuttle orally administered β -glucan to potentiate the CR3-dependent tumoricidal effects of monoclonal antibodies in mouse tumor models

Daniel J. Allendorf¹, Jarek T. Baran², Richard D. Hansen², Feng Hong³, Jose Marroquin², Jun Yan², Dick Walsh⁴, Gordon Ross¹. ¹Dept. of Microbiology/Immunology and James Graham Brown Cancer Center, University of Louisville, 529 S. Jackson St., Louisville, KY 40202, ²James Graham Brown Cancer Center, University of Louisville, Louisville, KY, ³Memorial Sloan-Kettering Cancer Center, New York, NY, ⁴Biopolymer Engineering, Eagan, MN

Intravenous administration of β -glucan has been shown previously to prime circulating leukocytes to migrate to and destroy iC3b-opsonized tumor cells in a CR3-dependent manner. Complement activation on tumor cells is a consequence of natural Ab or exogenous monoclonal Ab (mAb) recognizing tumor associated antigens (TAA). Combining mAb and oral β -glucan results in similar CR3-dependent tumor regression, but the priming of leukocyte CR3 is not immediate as in i.v. administration, requiring 2-3 days for activation of circulating neutrophils. After oral administration, fluorescein-conjugated glucan particles have been observed within M ϕ in lymph nodes, spleen, and bone marrow where large stores of neutrophils are found. Uptake of oral glucan particles by mucosal M ϕ was CR3 independent, but tumor regression was not observed in CR3 KO (CD11b^{-/-}) mice, implying CR3-dependence. Neutrophils, and perhaps M ϕ conveying glucan, migrate to the tumor where iC3b-opsonized tumor cells are destroyed. In mouse models, oral β -glucan in combination with i.v. mAb significantly decreased tumor burden and increased survival from subcutaneous and liver tumors. Regression occurs in SCID mice, indicating T cells are not required, however, oral β -glucan particles induced peritoneal M ϕ to produce IL-12 and circulating T cells to produce more IFN- γ and less IL-4 suggesting a shift from Th2 to Th1. Support: DOD DAMD 170210445

ting Abstracts

73.27

Tumor regression mediated by β -glucans against iC3b-opsonized tumors requires CR3⁺ neutrophils recruited via C5a and leukotriene B₄ (LTB₄)

Gordon D. Ross¹, Richard D. Hansen¹, Daniel J. Allendorf¹, Jarek T. Baran¹, Jun Yan¹, Krishnaprasad Subbarao¹, Bodduluri Haribabu¹, John D. Lambris². ¹James Graham Brown Cancer Center, University of Louisville, 529 S Jackson St, Louisville, KY 40202, ²Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA

Opsonization with iC3b is one of the major effector mechanisms of the C system against microorganisms. CR3 (CD11b/CD18) recognizes opsonic iC3b, but it must be deposited near a polysaccharide such as β -glucan that attaches to a lectin site in CR3. Because tumors lack such polysaccharides, iC3b-tumors do not stimulate phagocyte CR3-dependent cytotoxicity. Soluble β -glucans bind to the lectin site of CR3, priming it for cytotoxic degranulation in response to iC3b-opsonized tumors. When both anti-tumor mAb and soluble β -glucans are injected i.v. into mice with subcutaneous (s.c.) or liver tumors, the mAb activates the classical pathway and deposits iC3b on tumors which are then killed by CR3⁺ leukocytes. Soluble barley or particulate yeast β -glucans given orally mediate similar tumor regression that requires tumor-bound iC3b and fails in CR3^{-/-} (CD11b^{-/-}) mice. This study showed that s.c. tumors contained neutrophils but few macrophages, and that therapy failed in mice depleted of neutrophils with anti-Gr-1. A requirement for C5a- and LTB₄-mediated chemotaxis was indicated by therapy failures in both wild-type mice given a C5a-R-antagonist and in LTB₄-R^{-/-} mice. Thus, Ab mediated C activation deposits iC3b on tumors and releases C5a that likely stimulates LTB₄ release, the combination of which recruits neutrophils that kill the iC3b-targeted tumors via CR3.

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